

FIGS. 1-3
(Vasel Declaration)

Liquid Filled Projectiles



10%



25%



50%



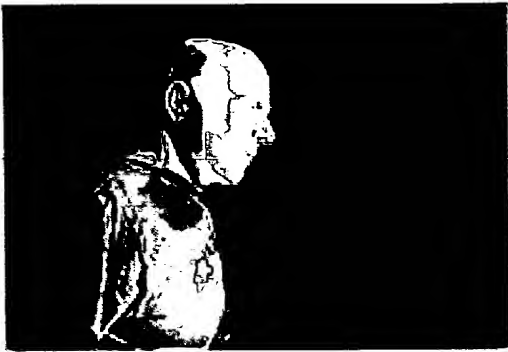
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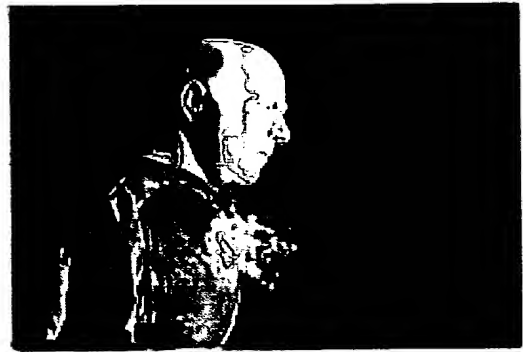
99%

Figure 1

Powder Filled Projectiles



10%



25%



40%



50%



75%



99%

Figure 2



Field Test



99%
Powder Filled



Figure 3

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Quantitative Analysis of Capsaicinoids in Fresh Peppers, Oleoresin Capsicum and Pepper Spray Products

REFERENCE: Reilly CA, Crouch DJ, Yost GS. Quantitative analysis of capsaicinoids in fresh peppers, oleoresin capsicum and pepper spray products. *J Forensic Sci* 2001;46(3):502-509.

ABSTRACT: Liquid chromatography-mass spectrometry was used to identify and quantify the predominant capsaicinoid analogues in extracts of fresh peppers, oleoresin capsicum, and pepper sprays. The concentration of capsaicinoids in fresh peppers was variable. Variability was dependent upon the relative pungency of the pepper type and geographical origin of the pepper. Nonivamide was conclusively identified in the extracts of fresh peppers, despite numerous reports that nonivamide was not a natural product. In the oleoresin capsicum samples, the pungency was proportional to the total concentration of capsaicinoids and was related by a factor of approximately 15,000 Scoville Heat Units (SHU)/ μ g of total capsaicinoids. The principle analogues detected in oleoresin capsicum were capsaicin and dihydrocapsaicin and appeared to be the analogues primarily responsible for the pungency of the sample. The analysis of selected samples of commercially available pepper spray products also demonstrated variability in the capsaicinoid concentrations. Variability was observed among products obtained from different manufacturers as well as from different product lots from the same manufacturer. These data indicate that commercial pepper products are not standardized for capsaicinoid content even though they are classified by SHU. Variability in the capsaicinoid concentrations in oleoresin capsicum-based self-defense weapons could alter potency and ultimately jeopardize the safety and health of users and assailants.

KEYWORDS: forensic science, pepper spray, capsaicin, LOMS

For centuries, people have used concentrated extracts of peppers (oleoresin capsicum) or the dried fruits to prepare spicy foods (1-4). Other historical and current uses of pepper products include neurobiological research (5), weight loss (6-8), local anesthesia (5,9), anti-microbial defense (10), anti-inflammation preparations (5,11), and recently for the production of self-defense and less-than-lethal (LTL) weaponry (3,12). The extensive use of peppers and pepper extracts for such diverse purposes emanates from the presence of capsaicinoids in the pepper.

The term "capsaicinoids" describes a group of pungent chemical analogues found in hot peppers (*Capsicum annuum* and *C. frutescens*) (1-4). There are five naturally occurring capsaicinoids: capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin (1-4). The most abundant and potent analogues in peppers (and consequently pepper extracts) are capsaicin and dihydrocapsaicin (1-4,13). Nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin are also present, but generally

contribute little to the total capsaicinoid concentration and pungency of the pepper (1-4,13). Nonivamide, or "synthetic" capsaicin, exhibits the same pungency as capsaicin, but has not been conclusively identified as a natural product (3,13). In fact, some scientists have concluded that detection of nonivamide in oleoresin capsicum was indicative of adulteration of the sample (3). The total concentration of capsaicinoids in a pepper ranges from 0.1 to 2.0% (dry weight) and depends upon the variety of the pepper, the growing conditions, and the time of harvest (1,3,4). In addition to differences in the total capsaicinoid content, variations in the relative proportions of the capsaicinoid analogues also occurs in response to the above criteria (1-4).

Capsaicinoids are the pharmacologically active and pain-producing components of the hot pepper (4). The characteristic chemical structure of capsaicin (or its analogues) contains a vanillamide moiety (4-hydroxy-3-methoxybenzylamide) and an acyl chain containing 10 to 11 carbon atoms (Fig. 1) (1-4,12). Capsaicinoids produce pain by stimulating the vanilloid receptor. This receptor is a molecular integrator of potentially noxious stimuli (e.g., low pH and high temperature) (5,11,14,15). Structure activity studies of capsaicin and other related compounds have demonstrated a strict requirement for the vanillyl ring and an acyl chain of 8 to 12 carbon atoms to manifest pungency (16-18). The natural capsaicinoids exhibit variable pungency due to differences in their ability to promote membrane depolarization through binding to the vanillamide receptor.

The ability of the capsaicinoids to produce pain has prompted the development of pepper sprays for the purpose of self-defense. Typically, pepper sprays weapons contain a 10% solution of oleoresin capsicum diluted in a suitable solvent (e.g., methylene chloride, trichloroethylene, isopropanol, freon(s), propylene glycol, ethanol, methanol, or dimethyl ether) and a gaseous propellant (usually N_2 or CO_2). Exposure to pepper sprays elicits an intense physiological response that includes nociception, temporary blindness, lacrimation, disorientation, shortness of breath, and choking (19). The result of the exposure is temporary incapacitation of the victim with minimal long-term side effects and/or toxicity (19,20).

Unfortunately, the manufacturers of oleoresin capsicum and self-defense weaponry employ few, if any, analytical measures to determine the concentration of active ingredients in the product and to ensure consistent chemical composition. Variability in the concentration of active ingredients might explain why pepper sprays have been shown to be only 70% effective in discouraging attacks by aggressive individuals (20). The principal test for product composition is a taste test to determine the product's ability to elicit

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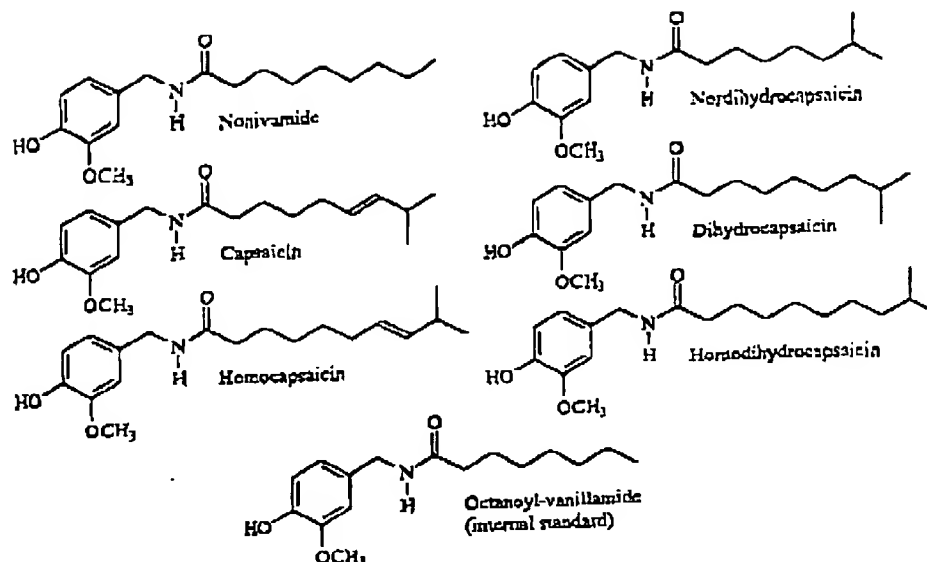


FIG. 1—Chemical structures of the capsaicinoid analogues and octanoyl-vanillamide (internal standard).

pain. This test is known as the Scoville Organoleptic Test (21). Unfortunately, the Scoville Organoleptic Test gives only a subjective measure of potency (SHU) rather than a quantitative assessment of the capsaicinoid concentration (21). Since the concentration of active ingredient in pepper products (including pepper sprays) is only subjectively assessed, the potency, efficacy, and potential product toxicity is difficult to predict (22).

In this study, we identified and quantified the individual capsaicinoid analogues in various fresh peppers, oleoresin capsicums, and pepper spray weapons. Nonivamide was identified in five different types of fresh peppers originating from different locations, as well as in all commercial pepper products. We also calculated the relationship between pungency and capsaicinoid concentration in oleoresin capsicum as well as in a series of pepper-based self-defense products.

Materials and Methods

Chemical

Extreme caution must be used when handling capsaicinoids and/or pepper products to prevent serious discomfort and/or injury. Capsaicin (60% purity), capsaicin (*E*-8-methyl-N-vanillyl-6-jur), dihydrocapsaicin (8-methyl-N-vanillyl-6-jur), and nonivamide (N-vanillyl-6-jur) were purchased from Sigma Chemical Co. (St. Louis, MO). Nordihydrocapsaicin, *E*-homocapsaicin, and homodihydrocapsaicin were purified from oleoresin capsicum using HPLC. Vanillamine hydrochloride and octanoyl chloride were purchased from Aldrich Chemical Company, Inc. (Milwaukee, WI). Octanoyl-vanillamide was synthesized by condensation of vanillamine and octanoyl chloride as previously described (12). HPLC-grade methanol and reagent-grade *n*-butyl chloride were purchased from Burdick and Jackson (Muskegon, MI). Formic acid, sodium phosphate, and sodium chloride were purchased from Mallinckrodt (Paris, KY). The oleoresin capsicum samples were obtained from various spice vendors and the pepper sprays were purchased from independent distributors.

Analytical Methods

Identification and quantification of the individual capsaicinoids was performed by liquid chromatography-mass spectrometry using a Hewlett-Packard series 1100 LC/MSD (Agilent Technologies, Palo Alto, CA). The capsaicinoids were separated using a MetaSil Basic reversed-phase HPLC column (100 × 3.0 × 3 μm) (MetaChem Technologies, Torrance, CA) and a stepwise gradient of methanol and distilled water containing 0.1% (v/v) formic acid. The mass spectrometer was set to detect the *M*+*H* positive ions of octanoyl-vanillamide (*m/z* = 280), nonivamide (*m/z* = 294), nordihydrocapsaicin (*m/z* = 294), capsaicin (*m/z* = 306), dihydrocapsaicin (*m/z* = 308), homocapsaicin (*m/z* = 320), and homodihydrocapsaicin (*m/z* = 322).

Preparation of the analytical standards was achieved by weighing the appropriate quantity of each analogue on a Cahn 4700 analytical balance (Cahn Instruments, Cerritos, CA) and dissolving the compounds in methanol. Standards were stored at -20°C and were stable for the duration of the study. Calibration curves, containing all analogues and 500 ng octanoyl-vanillamide (as an internal standard), were constructed from 0 to 5000 ng. Calibration curves were generated by calculating the peak area ratio (obtained using HP Chemstation software) of the analyte to internal standard. Linear regression analyses were performed using the least squares method. Separate curves were used to quantify the low- (1 to 50 ng), mid- (50 to 500 ng), and high- (500 to 5000 ng) range concentrations of the analogues. Split curves were needed to more accurately quantify the concentration of the compounds in the samples.

Analysis of Fresh Peppers

Extracts of fresh peppers were prepared by homogenizing approximately 5 to 50 g of fresh pepper (purchased from food stores in UT, NM, and MD) in 5 volumes of phosphate-buffered saline (50 mM phosphate buffer, pH 7.2 containing 1 M NaCl) using a Teflon/glass homogenizer. To decrease the sample size of the fresh peppers, the stem and a portion of the pericarp were removed; the stem and pericarp have been shown to contain only negligible con-

centrations of the capsaicinoids (1,23,24). The homogenate was extracted for 15 min at room temperature with *n*-butyl chloride. The extract was centrifuged at $1000 \times g$ for 10 min and the upper organic layer was collected. The extraction process was repeated and the organic layers were combined and evaporated to dryness under a stream of air at 40°C . The dried residues were reconstituted in $200 \mu\text{L}$ *n*-butyl chloride:methanol (1:1). The reconstituted residues were diluted 20-fold in *n*-butyl chloride:methanol (1:1) and a $5 \mu\text{L}$ aliquot was combined with 500 ng octanoyl-vanillamide. The samples were evaporated to dryness, reconstituted with $100 \mu\text{L}$ of 70% methanol: 30% distilled H_2O , and analyzed using LC/MS.

Analysis of Oleoresin Capsicum

Commercially available oleoresin capsicum samples were diluted 200-fold in *n*-butyl chloride:methanol (1:1). A $5 \mu\text{L}$ aliquot of the diluted product was pipetted into a 13×100 mm silanized glass tube and octanoyl-vanillamide (500 ng) was added as the internal standard. The sample was evaporated to dryness, reconstituted with $100 \mu\text{L}$ of 70% methanol: 30% distilled H_2O , and analyzed using LC/MS.

Analysis of Pepper Spray

Individual pepper spray canisters were vigorously shaken and cooled to -20°C overnight in a freezer. The sprays were then gently discharged into a 16×100 silanized glass tube that had been previously equilibrated to -80°C using dry ice. Cooling the tubes

with dry ice was necessary to prevent evaporation of the solvent during collection. The sample was immediately capped and thawed on ice. The sample volume was determined and the volatile components were permitted to evaporate at room temperature with gentle agitation. The sample volume was reestablished by adding *n*-butyl chloride:methanol (1:1). Samples were then diluted 40-fold in *n*-butyl chloride:methanol (1:1) and a $5 \mu\text{L}$ aliquot combined with octanoyl-vanillamide (500 ng). The samples were evaporated to dryness, reconstituted with $100 \mu\text{L}$ of 70% methanol: 30% dH_2O , and analyzed using LC/MS.

Results

Analysis of Capsaicinoids by LC/MS

Identification of the individual capsaicinoid analogues (see Fig. 1) was achieved by LC/MS. The calibration curve for the assay was linear from 1.0 to 5000 ng, but was split into three curves to more accurately calculate the concentration of the analytes in the samples. Identification of the individual capsaicinoids was achieved by mass-selective detection as well as by retention time. Octanoyl-vanillamide ($m/z = 280$) eluted at approximately 8.4 min followed by nordihydrocapsaicin ($m/z = 294$; 11.8 min), nonivamide ($m/z = 294$; -12.6 min), capsaicin ($m/z = 306$; -12.6 min), dihydrocapsaicin ($m/z = 308$; -15.8 min), homocapsaicin ($m/z = 320$; -16.1 min), and homodihydrocapsaicin ($m/z = 322$; -17.5 min). A typical mass-chromatogram of a standard containing 500 ng of all analytes and internal standard is shown in Fig. 2. The compound

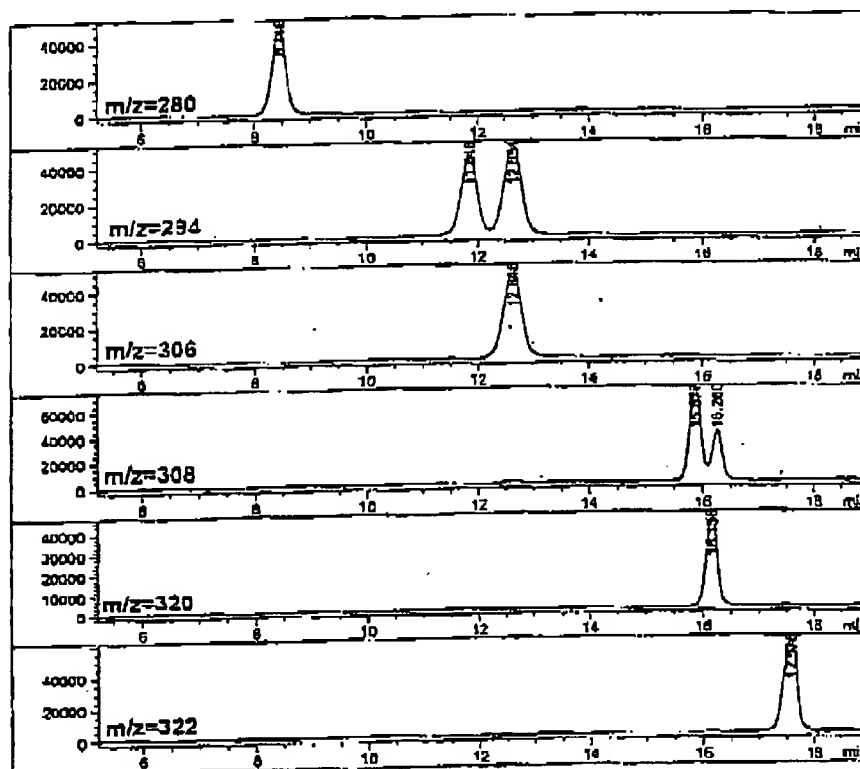


FIG. 2—Representative reconstructed ion mass-chromatogram of a 500 ng standard mixture containing all capsaicinoids and internal standard (500 ng). Octanoyl-vanillamide is represented by the panel labeled $m/z = 280$, nordihydrocapsaicin and nonivamide by $m/z = 294$, capsaicin by $m/z = 306$, dihydrocapsaicin by $m/z = 308$, homocapsaicin by $m/z = 320$, and homodihydrocapsaicin by $m/z = 322$. Specific retention times for the individual analytes are presented in the results section. Data were collected as described under materials and methods.

(*m/z* = 308) eluting at approximately 16.2 min has been tentatively identified as decanoyl-vanillamide, based on similarities in retention time and mass when compared to a synthetic standard of decanoyl-vanillamide (data not shown).

Analysis of Capsaicinoids in Fresh Peppers

The analysis of "unadulterated" pepper extracts demonstrated the presence of all previously documented naturally occurring capsaicinoids as well as nonivamide. The identification of nonivamide in peppers was confirmed by mass (*m/z* = 294) and retention time (approximately 12.6 min). Confirmation of the presence of nonivamide in the peppers was achieved using collision-induced fragmentation of the molecular ion by LC/MS/MS and obtaining the product ion spectrum (data not shown). Nonivamide was present in habanero, anaheim, red-chili, green-chili, and green bell pepper extracts at a relative concentration range of 0.42 to 7.2% of the total capsaicinoid concentration, depending upon the variety of pepper. Nonivamide was not detected in extracts of yellow bell pepper and was present in the green bell pepper at less than 0.025% of the concentration in green-chili pepper. The concentrations of the capsaicinoid analogues in the six varieties of peppers are summarized in Table 1.

cinoid analogues in the six varieties of peppers are summarized in Table 1.

Analysis of Oleoresin Capsicum

As described, quantification of the individual capsaicinoid analogues in commercial preparations of oleoresin capsaicum was achieved by comparing the peak area ratio of the samples to the calibration curves. The capsaicinoid analogue concentrations in seven different oleoresin capsaicum samples are summarized in Table 2. The absolute and relative concentrations of the individual capsaicinoids varied between samples. Figures 3A and 3B illustrate the correlation between SHU rating and the total (Fig. 3A) and individual capsaicinoid analogue concentrations (Fig. 3B). The relationship between total capsaicinoid concentration and SHU rating was approximately 15 000 SHU/ μg of total capsaicinoids. Data presented in Fig. 3B indicate that capsaicin and dihydrocapsaicin were primarily responsible for the SHU rating. Their relationships were approximately 35 700 and 33 900 SHU/ μg , respectively. The total and relative analogue concentrations were consistent between oleoresin capsaicum samples having the same SHU rating.

TABLE 1—Capsaicinoid analogue concentrations in extracts of fresh peppers.

Variety of Pepper	Total ($\mu\text{g/g}$) ^a	NDHC† (%)	Nonivamide (%)	Capsaicin (%)	DHC† (%)	HC† (%)	HDHC† (%)
Yellow Bell (Utah)	0.0018 \pm 0.0002	N.D.	N.D.	37 \pm 4.0	63 \pm 5.0	N.D.	N.D.
Green Bell (Utah)	0.0049 \pm 0.0004	13.1 \pm 0.9	7.2 \pm 0.4	27 \pm 2.0	52 \pm 7.0	N.D.	N.D.
Green-chili (Utah)	19 \pm 2.0	22.4 \pm 0.2	2.7 \pm 0.2	21 \pm 6.0	36 \pm 2.0	0.9 \pm 0.4	7 \pm 2.0
Anaheim (New Mexico)	73 \pm 7.0	11.8 \pm 0.9	1.0 \pm 0.1	47 \pm 2.0	36 \pm 3.0	1.3 \pm 0.4	3.0 \pm 0.7
Anaheim (Maryland)	87 \pm 5.0	6.3 \pm 0.8	0.42 \pm 0.07	60 \pm 9.0	30 \pm 4.0	2.6 \pm 0.5	1.4 \pm 0.3
Red-Chili (New Mexico)	83 \pm 9.0	11 \pm 1.0	0.6 \pm 0.1	56 \pm 4.0	29 \pm 2.0	1.0 \pm 0.5	1.8 \pm 0.4
Red-chili (Utah)	59 \pm 6.0	9.6 \pm 0.4	1.2 \pm 0.1	50 \pm 3.0	27 \pm 1.0	6.1 \pm 0.4	6.2 \pm 0.5
Habanero (Utah)	510 \pm 27.0	3.6 \pm 0.3	1.4 \pm 0.1	61 \pm 6.0	32 \pm 3.0	0.9 \pm 0.1	1.1 \pm 0.1

^a Approximately 5 to 50 g of fresh pepper was extracted as described under materials and methods. Data are representative of the mean \pm standard deviation of triplicate analysis of pepper extracts.

† Abbreviations for Nordihydrocapsaicin (NDHC), Dihydrocapsaicin (DHC), Homocapsaicin (HC), and Homodihydrocapsaicin (HDHC).

TABLE 2—Capsaicinoid analogue concentrations for various oleoresin capsaicum samples.

Sample Identity†	Total ($\mu\text{g/mL}$) ^a	NDHC† (%)	Nonivamide (%)	Capsaicin (%)	DHC† (%)	HC† (%)	HDHC† (%)
OC1	0.8 \pm 0.2	16.7 \pm 0.3	1.8 \pm 0.4	38 \pm 1.0	36 \pm 2.0	7.0 \pm 3.0	N.D.
OC2	14 \pm 1.0	20 \pm 2.0	1.9 \pm 0.2	36 \pm 4.0	38 \pm 3.0	2.0 \pm 0.1	1.9 \pm 0.2
OC3	18.4 \pm 0.4	18.7 \pm 0.3	1.2 \pm 0.9	35.9 \pm 0.5	39.3 \pm 0.4	2.0 \pm 0.1	1.9 \pm 0.4
OC4	63 \pm 1.0	11 \pm 1.0	3.4 \pm 0.5	33 \pm 2.0	48 \pm 3.0	1.4 \pm 0.2	2.9 \pm 0.7
OC5	71 \pm 2.0	8.6 \pm 0.7	5.2 \pm 0.8	39 \pm 3.0	42 \pm 1.0	1.5 \pm 0.1	2.4 \pm 0.3
OC6	67 \pm 3.0	6.3 \pm 0.4	5.5 \pm 0.2	47.8 \pm 0.9	35.8 \pm 0.7	1.7 \pm 0.8	2.1 \pm 0.1
OC7	131 \pm 5.0	7.7 \pm 0.1	4.2 \pm 0.1	41.2 \pm 0.5	43.5 \pm 0.9	1.3 \pm 0.2	2.3 \pm 0.4

^a Data are representative of the mean \pm standard deviation of triplicate analysis of the oleoresin capsaicum sample.

† Abbreviations for Nordihydrocapsaicin (NDHC), Dihydrocapsaicin (DHC), Homocapsaicin (HC), and Homodihydrocapsaicin (HDHC).

[‡] Represents oleoresin capsaicum samples with the following SHU ratings: OC1 (2.0×10^4 SHU), OC2 (2.0×10^5 SHU), OC3 (5.0×10^5 SHU), OC4-6 (1.0×10^6 SHU), OC7 (2.0×10^6 SHU). Although OC4-6 have the same SHU rating, they were obtained from different manufacturers of oleoresin capsaicum.

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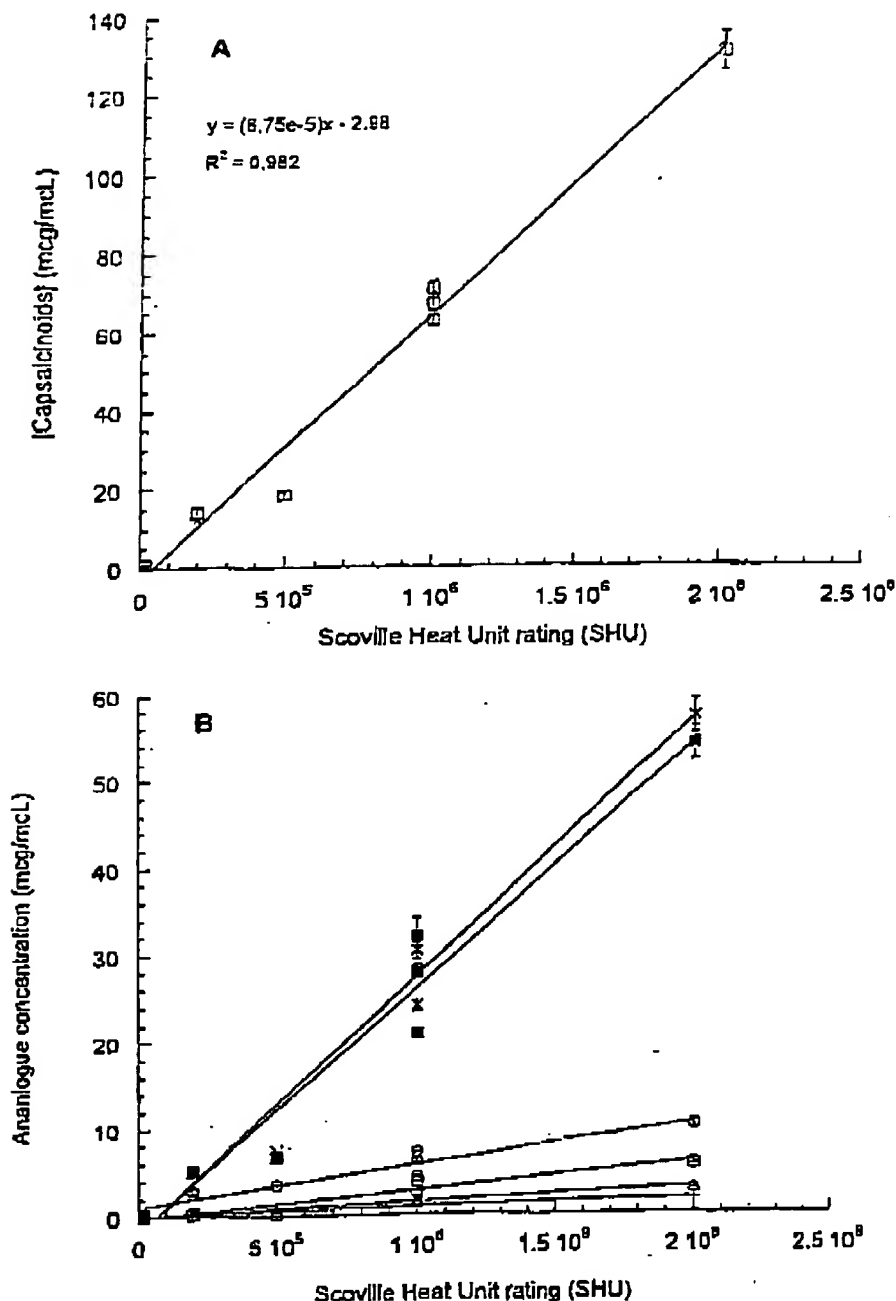


FIG. 3—(A) The relationship between Scoville Heat Unit rating and total capsaicinoid concentration in oleoresin capicum, (B) The relationship between Scoville Heat Unit rating and the concentration of individual capsaicinoid analogues in oleoresin capicum; (O) nordihydrocapsaicin ($y = 4.7 \times 10^{-6}x + 1.0$), (□) nonivamide ($y = 3.17 \times 10^{-6}x + 0.3$), (Δ) homodihydrocapsaicin ($y = 1.5 \times 10^{-6}x + 0.02$), (■) capsaicin ($y = 2.8 \times 10^{-5}x + 1.9$), (X) dihydrocapsaicin ($y = 2.95 \times 10^{-5}x + 2.0$), and (+) homocapsaicin ($y = 8.5 \times 10^{-7}x + 0.1$). Data represent the mean \pm standard deviation of triplicate samples. Analysis of the capsaicinoids in oleoresin capicum was performed as described under materials and methods.

Analysis of Capsaicinoids in Pepper Spray

Total and individual capsaicinoid analogue concentrations for several commercially available pepper spray products are summarized in Table 3. The total capsaicinoid concentrations were consistent with previous reports of the composition of pepper sprays (25). The capsaicinoid concentrations in pepper spray products from dif-

ferent product lots from the same manufacturer are also presented in Table 3. A comparison between the labeled and calculated SHU values for the pepper sprays is shown in Table 4. Collectively, these data demonstrate that there were substantial differences in the total and relative capsaicinoid concentrations in the pepper spray products. Also, the labeled SHU values of the pepper sprays were not consistent with the assayed values.

TABLE 3—Capsaicinoid analogue concentrations of various pepper sprays.

Sample Identity	Total ($\mu\text{g}/\mu\text{L}$) ^a	NDHC† (%)	Nonivamide (%)	Capsaicin (%)	DHC† (%)	HC† (%)	HDHC† (%)
PS1‡	16.0 ± 0.4	6.4 ± 0.2	1.6 ± 0.1	51 ± 1.0	37.1 ± 0.8	2.2 ± 0.1	1.5 ± 0.1
PS2‡	11.8 ± 0.4	8.7 ± 0.3	2.0 ± 0.1	45 ± 2.0	40 ± 1.0	2.0 ± 0.1	2.1 ± 0.1
PS3	0.95 ± 0.03	5.7 ± 0.2	1.8 ± 0.1	52.4 ± 0.9	37 ± 1.0	2.4 ± 0.3	0.8 ± 0.1
PS4	8.3 ± 0.4	0.2 ± 0.01	1.9 ± 0.1	50 ± 3.0	35 ± 1.0	1.6 ± 0.1	2.0 ± 1.0
PS5§	2.5 ± 0.2	9.7 ± 0.6	1.9 ± 0.1	41 ± 3.0	41 ± 3.0	2.2 ± 0.2	2.5 ± 0.9
PS6§	13.5 ± 0.1	10.8 ± 0.1	1.8 ± 0.1	36.5 ± 0.2	45.8 ± 0.3	1.8 ± 0.1	2.0 ± 0.8
PS7	13 ± 1.0	9.6 ± 0.9	2.2 ± 0.3	42 ± 3.0	42 ± 4.0	1.8 ± 0.2	1.9 ± 0.8
PS8	27 ± 3.0	N.D.	100 ± 10.0	N.D.	N.D.	N.D.	N.D.
PS9	32 ± 1.0	N.D.	100 ± 4.0	N.D.	N.D.	N.D.	N.D.

^a Total capsaicinoid concentrations ($\mu\text{g}/\mu\text{L}$) of dispensed pepper spray. This value does not represent the total capsaicinoid content in the canister. Data are representative of the mean ± standard deviation of triplicate analysis of the pepper spray product.

† Abbreviations for Nordihydrocapsaicin (NDHC), Dihydrocapsaicin (DHC), Homocapsaicin (HC), and Homodihydrocapsaicin (HDHC).

‡, §, || Represents pepper sprays from the same manufacturer, but different product lots.

TABLE 4—Calculated versus labeled SHU value for various pepper spray products.

Sample Identity	Total ($\mu\text{g}/\mu\text{L}$) ^a	Labeled SHU	Calculated SHU
PS1	16.0 ± 0.4	2.0×10^6	2.4×10^5
PS2	11.8 ± 0.4	2.0×10^6	1.8×10^5
PS3	0.95 ± 0.03	2.0×10^6	1.5×10^4
PS4	8.3 ± 0.4	2.0×10^6	1.3×10^4
PS5	2.5 ± 0.2	1.5×10^6	3.7×10^4
PS6	13.5 ± 0.1	1.5×10^6	2.0×10^5
PS7	13 ± 1.0	1.5×10^6	1.9×10^5
PS8†	27 ± 3.0	1.0×10^6	9.0×10^5
PS9†	32 ± 1.0	1.0×10^6	1.0×10^6

^a Data are representative of the mean ± standard deviation of triplicate analysis of the pepper spray products.

† Calculated SHU values were determined using the formula $\text{SHU} = [\text{capsaicin}] (\mu\text{g}/\mu\text{L}) \times 33,000 (\text{SHU}/\mu\text{g})$ since only nonivamide was present (nonivamide has similar pungency to capsaicin (13) and the concentration was not in range for the trend line for nonivamide).

Discussion

The data demonstrate that capsaicin, dihydrocapsaicin, nordihydrocapsaicin, homocapsaicin, homodihydrocapsaicin, and nonivamide can be found in fresh peppers, oleoresin capsicum, and pepper spray products. However, the total and relative concentrations of the capsaicinoid analogues in the peppers and the pepper products were quite variable (Tables 1 to 3). In all samples, capsaicin and dihydrocapsaicin constituted 60 to 90% of the total capsaicinoid concentration, followed by nordihydrocapsaicin (2 to 20%), homocapsaicin (1 to 5%), homodihydrocapsaicin (1 to 5%), and nonivamide (1 to 5%).

Capsaicin, nonivamide, and dihydrocapsaicin are the most pungent capsaicinoid analogues having 100, 100, and 75% relative pungencies, respectively (12,13,18). Nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin exhibit relative pungencies that range between 20 to 50% that of capsaicin (12,13,18). Because nordihydrocapsaicin, homocapsaicin, and homodihydrocapsaicin contribute little to the relative pungency of the peppers or pepper products, their contribution to pungency is not discussed below. However, this does not imply that these analogues may not be important in the toxicological and/or pharmacological properties of the products.

To determine the source of variability in pepper sprays and oleoresin capsicum, we first analyzed the capsaicinoid concentrations in fresh peppers. In the extracts of fresh peppers, the total concentration of the capsaicinoids, as well as that of each analogue, was variable and dependent upon the variety and geographical origin of the pepper. The total capsaicinoid concentration in extracts of the fresh peppers was reflective of the relative "hotness" of the pepper (habanero > anaheim = red-chili > green-chili > green bell > yellow bell). Capsaicin and dihydrocapsaicin were the most abundant capsaicinoid analogues present in the extracts of fresh peppers and comprised 60 to 90% of the total capsaicinoid concentration. The sum of the concentrations of capsaicin and dihydrocapsaicin in the extracts of fresh pepper paralleled the relative pungency of the pepper type and appeared to dictate the pungency of the fruit (see Table 1). For example, extracts of habanero peppers (the "hottest" pepper) contained approximately 90% capsaicin and dihydrocapsaicin while extracts of the milder green-chili pepper contained about 65% capsaicin and dihydrocapsaicin.

The immediate precursor to pepper sprays is oleoresin capsicum, the extracted product of hot peppers. As expected, we also observed variability in the total and relative concentrations of the capsaicinoids in commercial preparations of oleoresin capsicum and the SHU rating of the product (Fig. 3A). Comparing the individual capsaicinoid analogue concentrations to SHU rating demonstrated that the concentrations of capsaicin and dihydrocapsaicin were good predictors of the pungency of the oleoresin capsicum sample (Fig. 3B). For example, oleoresins having an SHU rating $\leq 5.0 \times 10^5$ SHU contained approximately 65% capsaicin and dihydrocapsaicin and samples with an SHU rating $\geq 1.0 \times 10^6$ consisted of >80% capsaicin and dihydrocapsaicin.

Since both fresh peppers and oleoresin capsicum exhibited variability in the concentrations of the capsaicinoids, we were not surprised to find significant differences in the total and relative concentrations of capsaicinoids of the pepper sprays. An interesting discovery was that variability was present in all commercially available pepper sprays even in samples from the same manufacturer (Table 3). For example, two identical pepper spray products from different lots and the same manufacturer exhibited striking differences in total capsaicinoid concentration, $2.5 \pm 0.2 \mu\text{g}/\mu\text{L}$ versus $13.5 \pm 0.1 \mu\text{g}/\mu\text{L}$. Variability in product composition was also observed in products from different manufacturers that were both labeled as 10% oleoresin capsicum with a rating of 2.0×10^6 .

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SHU. The two products had vastly different capsaicinoid concentrations ($0.95 \pm 0.03 \mu\text{g}/\mu\text{L}$ versus $16.0 \pm 0.4 \mu\text{g}/\mu\text{L}$). Estimating the SHU value based on the total capsaicinoid concentration of the pepper sprays suggested that the labeled SHU values were sometimes overstated by a factor of >100 times (see Table 4).

Trends suggesting that the relative analogue concentrations of capsaicin and dihydrocapsaicin were responsible for the SHU rating of the product were apparent for both fresh peppers and oleoresin capsicum samples. In pepper sprays, the relative concentrations of capsaicin and dihydrocapsaicin were $>80\%$. This may imply that the majority of the pepper sprays may have been prepared as dilutions of oleoresin capsicum having an SHU rating $\approx 1.0 \times 10^6$. Unfortunately, dilution of oleoresin capsicums having similar SHU rating does not appear to guarantee consistency in the amount of active ingredient in the pepper spray. Therefore, the variability in the capsaicinoid concentrations of the pepper sprays can be attributed to the inherent variability in the capsaicinoid concentrations of different batches of oleoresin capsicum and ultimately the particular properties of the extracted peppers.

From our data, it is reasonable to conclude that manufacturers of oleoresin capsicum-based pepper sprays do not standardize their product for the concentration of capsaicinoids. This was not true for samples PS8 and PS9 (Table 4). The manufacturer of this product used a known quantity of nonivamide as the active ingredient rather than a standard volume of oleoresin capsicum. Previous work has demonstrated that nonivamide exhibits identical pungency to capsaicin based upon the Scoville Organoleptic Test (12,13,18). It also has a similar structure activity relationship using desensitization of the VR receptor as a model system to predict pungency (12–18). Therefore, products fortified with or composed solely of nonivamide are likely to have similar efficacy to oleoresin capsicum-based pepper sprays for use as self-defense weapons. However, the studies reported here did not evaluate or compare the biological efficacy of products.

Nonivamide is not commonly used to manufacture pepper sprays because it is considered a "synthetic" and/or pharmaceutical agent. Oleoresin capsicum is a natural product. However, pepper sprays manufactured from oleoresin capsicum are not regulated. Thus far, the identification of nonivamide in fresh peppers has not been definitively confirmed and its existence as a natural product debated (3). Some authors suggested that nonivamide did not occur naturally because of the structure of its acyl chain (3). This argument was supported by inconclusive analytical data (3). GC/MS methods were ineffective at differentiating nordihydrocapsaicin and nonivamide since they have the same molecular weight (3,26–31). Standard HPLC methods did not chromatographically separate capsaicin from nonivamide (3, 31–35). The method described here allowed us to uniquely identify and quantify nonivamide in extracts of fresh pepper, oleoresin capsicum, and pepper sprays. Nonivamide was present at a concentration that represented up to 7.2% of the total capsaicinoid concentration (Tables 1–3).

Based on our work, several conclusions about the concentrations of capsaicinoids in peppers as well as products manufactured from extracts of peppers can be made. Because oleoresin capsicum is obtained by extracting fresh peppers, variability in the total and relative capsaicinoid concentrations exists. As such, products made from the oleoresin capsicum (e.g., food products, pepper sprays, etc.) also exhibit variability. Differences in the concentrations of active components in pepper sprays may affect the quality, efficacy, and safety of these products (20,37,38). Discrepancies in the amount of active ingredient in the products may result in unpre-

dictable results when the products are used for self-defense or to subdue suspects. Differences in the concentration of active ingredients of the products could also affect the potential toxicity of the products and could jeopardize the safety and health of individuals who are exposed to and/or depend upon the products for protection (20,37–39). Quantitative analytical methods are needed to determine the concentration of capsaicinoids in pepper sprays. Implementing this objective quality control measure, as well as regulating the formulation of pepper sprays, would substantially increase the predictability of product potency, efficacy, and its potential to cause toxicity.

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